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DATE: Friday, January 31, 2003 Printable Copy Create Case

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<u>L3</u>	<u>3</u>	11 with L2	1326	<u>L3</u>
<u>L2</u>	2	translation\$	174996	<u>L2</u>
<u>L</u> 1	<u>[</u>	signal sequence	28365	<u>L1</u>

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09/829251 Att 29

=> s signal(w)sequence L1 24196 SIGNAL(W) SEQUENCE => s translation? L2 468945 TRANSLATION? => s 11(5n)I2 287 L1(5N) L2 => s 11(1)122667 L1(L) L2 => s variant or mutant or mutat? L5 1460238 VARIANT OR MUTANT OR MUTAT? => s 11(5n)15722 L1(5N) L5 => s 16 and 12 114 L6 AND L2 1.7 => dup rem 13 PROCESSING COMPLETED FOR L3 135 DUP REM L3 (152 DUPLICATES REMOVED) => dup rem 17 PROCESSING COMPLETED FOR L7 56 DUP REM L7 (58 DUPLICATES REMOVED) => s 18 and py<1996 2 FILES SEARCHED... 4 FILES SEARCHED... 92 L8 AND PY<1996 => s 19 and ly<1996 '1996' NOT A VALID FIELD CODE 0 L9 AND LY<1996 => s 110 or 111 1.12 92 L10 OR L11 => s 19 and py<1996 1 FILES SEARCHED... 3 FILES SEARCHED... 4 FILES SEARCHED... 35 L9 AND PY<1996 => s 110 or 113 123 L10 OR L13 => d 114 ibib abs I-123 L14 ANSWER LOF 123 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. ACCESSION NUMBER: 1996:60002 BIOSIS DOCUMENT NUMBER: PREV199698632137 Molecular cloning of a novel myeloid granule protein. Moscinski, Lynn C. (1); Hill, Bobbye AUTHOR(S): CORPORATE SOURCE: (1) Dep. Pathol., H. Lee Moffitt Cancer Center Res. Inst., 12902 Magnolia Drive, Tampa, FL 33612 USA SOURCE: Journal of Cellular Biochemistry, (1995) Vol. 59, No. 4, pp. 431-442. ISSN: 0730-2312. DOCUMENT TYPE: Article LANGUAGE: English AB Granulocytes are recognized by the presence of granules, including

(azurophilic) and secondary types. Each granule type contains distinct and

characteristic families of enzymes. We have screened a murine bone marrow

cDNA library to obtain a series of sequences corresponding to mRNAs which

are both myeloid-specific and appear to be expressed only in immature bone

marrow cells. A 1,160 bp sequence (B9) has been isolated, which shows restricted expression in murine bone marrow, with the highest levels in cultures enriched for promyelocytes. Translation yields a single open reading frame of 167 amino acids and a calculated MW of 19.33 kd. A note.

potential N-glycosylation site is present. Evaluation of the amino terminal sequence shows 2 polar amino acids flanking a hydrophobic region,

suggesting a \*\*\*signal\*\*\* \*\*\*sequence\*\*\* and the possibility of post- \*\*\*translational\*\*\* modification. An extensive search of the protein data base reveals 30% identity over 90 amino acids with porcine cathelin, a cystatin-like cysteine proteinase inhibitor. This sequence identity includes conservation of the 4 cysteine residues noted in all members of the cystatin superfamily. In an attempt to further characterize this novel sequence, a polyclonal antiserum was prepared by immunization with a 20 amino acid synthetic peptide corresponding to a unique portion of the carboxy terminus. Immunoelectron microscopy localized B9 to neutrophilic granules. We have identified a novel myeloid-specific granule protein related to porcine cathelin, but showing important structural differences. This may represent the first isolated member of a new cystatin family. More importantly, the small size of the B9 gene and its tight pattern of early expression make B9 an excellent reporter molecule for the study of new factors important in myeloid differentiation.

L14 ANSWER 2 OF 123 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1996:35560 BIOSIS DOCUMENT NUMBER: PREV199698607695

TITLE: Structure, organization, and transcription units of the human alpha-platelet-derived growth factor receptor gene, PDGFRA.

AUTHOR(S): Kawagishi, Jun; Kumabe, Toshihiro; Yoshimoto, Takashi:

Yamamoto, Tokuo (1)

CORPORATE SOURCE: (1) Tohoku Univ. Gene Res. Cent., 1-I Tsutsumi-dori-

Amamiya, Aoba, Sendai 981 Japan

SOURCE: Genomics, (1995) Vol. 30, No. 2, pp. 224-232. ISSN: 0888-7543.

DOCUMENT TYPE: Article
LANGUAGE: English

AB Isolation and characterization of genomic clones encoding human alpha-platelet derived growth factor receptor (HGMW-approved symbol PDGFRA) revealed that the gene spans approximately 65 kb and contains

exons. The 5'-untranslated region of the mRNA is encoded by exon 1, and

large intron of 23 kb separates exon 2 encoding the \*\*\*translation\*\*\* initiator codon AUG and the \*\*\*signal\*\*\* \*\*\*sequence\*\*\*. The locations of exon/intron boundaries in the extracellular immunoglobulin-like domains, the transmembrane domain, the two cytoplasmic

tyrosine kinase domains, and the kinase insertion domain are very similar to those in c-kit and macrophage colony stimulating factor-1 receptor genes. The transcription start site was mapped to a position 393 hp upstream of the AUG translation initiator codon by S1 mapping and inter-

extension analysis. The 5'-flanking region of the gene lacks a typical TATA box but contains a typical CCAAT box and GATA motifs. This region

also contains potential sites for AP-1, AP-2, Oct-1, Oct-2, and Sp1. The 5'-flanking region of the gene was fused to the luciferase reporter gene, and transcription units of the gene were determined.

L14 ANSWER 3 OF 123 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1995:483885 BIOSIS DOCUMENT NUMBER: PREV199598498185

TITLE: Characterization of a new rho mutation that relieves polarity of Mu insertions.

AUTHOR(S): Peters, Joseph E.; Benson, Spencer A. (1)
CORPORATE SOURCE: (1) Dep. Microbiol., Univ. Md. College Park,

09/829251 AHAZY

=> s stii or st I l

567 STII OR STII

=> s signal sequence?

27755 SIGNAL SEQUENCE?

=> s secretion signal

1825 SECRETION SIGNAL L3

=> s 12 or 13

L4 28950 L2 OR L3

=> s 11 and 14

L5 59 L1 AND L4

=> dup rem 15

PROCESSING COMPLETED FOR L5

27 DUP REM L5 (32 DUPLICATES REMOVED)

=> s 16 and py<1996

1 FILES SEARCHED...

3 FILES SEARCHED ...

4 FILES SEARCHED...

15 L6 AND PY<1996

=> d 17 ibib abs 1-15

L7 ANSWER I OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL

ABSTRACTS INC.

ACCESSION NUMBER: 1993:436618 BIOSIS DOCUMENT NUMBER: PREV199396091243

TITLE: Expression, purification, and characterization of

recombinant ornatin E, a potent glycoprotein IIb-IIIa

antagonist.

AUTHOR(S): Mazur, Paul: Dennis, Mark S.; Seymour, Jana L.;

Lazarus,

Robert A. (1)

CORPORATE SOURCE: (1) Dep. Protein Eng., Genentech Inc., South San

Francisco.

CA 94080 USA

SOURCE: Protein Expression and Purification, (1993) Vol. 4, No. 4,

pp. 282-289.

ISSN: 1046-5928.

DOCUMENT TYPE: Article LANGUAGE: English

AB A synthetic gene encoding ornatin E (OrnE), a 50-amino acid

glycoprotein

IIb-IIIa (GP IIb-IIIa) antagonist and platelet aggregation inhibitor isolated from the leech Placobdella ornata, was designed, constructed, and expressed in Escherichia coli. The OrnE gene was fused to the heat stable enterotoxin \*\*\*stl|\*\*\* \*\*\*signal\*\*\* \*\*\*sequence\*\*\* and expressed under the transcriptional control of the E. coli alkaline phosphatase promoter. This construction directed secretion of recombinant ornatin E (rOmE) into the extracellular medium at levels of 7-19 mg/liter. The protein was purified to apparent homogeneity in 18-38% yields by ammonium sulfate precipitation, Q-Sepharose and S-Sepharose ion

exchange chromatography, and reverse-phase HPLC. Purified rOmE was

to be indistinguishable from leech-derived OrnE as judged by amino acid composition, N-terminal sequencing, mass spectroscopic analysis, and **HPLC** 

coelution. In addition, rOrnE exhibits similar activity in fibrinogen/GP 11b-111a ELISA and platelet aggregation assays. Purified rOmE possesses three disulfide bonds, the reduction and carboxymethylation of which results in a ca. 60-fold reduction in biological activity. A misfolded variant of rOrnE was characterized and shown to have a ca. 6-fold reduction in activity. These data demonstrate that the native disulfide bonds are required for the optimal GP IIb-IIIa antagonist activity of the omatins.

L7 ANSWER 2 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1993:203155 BIOSIS

DOCUMENT NUMBER: PREV199395104380

TITLE: Binding interactions of kistrin with platelet glycoprotein IIb-IIIa: Analysis by site-directed mutagenesis.

Dennis, Mark S.; Carter, Paul; Lazarus, Robert A. (1) AUTHOR(S): CORPORATE SOURCE: (1) Dep. Protein Eng., Genentech Inc., 460 Point

San Bruno

Blvd., South San Francisco, CA 94080 USA

SOURCE: Proteins Structure Function and Genetics, (1993) Vol. 15,

> No. 3, pp. 312-321. ISSN: 0887-3585.

DOCUMENT TYPE: Article LANGUAGE: English

AB The binding interactions between platelet fibrinogen receptor, glycoprotein (GP) IIb-IIIa, and kistrin, a snake venom disintegrin protein that contains the adhesion site recognition sequence Arg-Gly-Asp (RGD)

potently inhibits platelet aggregation, have been investigated by site-directed mutagenesis of a synthetic kistrin gene. Kistrin was expressed as a fusion protein in Escherichia coli under control of the alkaline phosphatase promoter. This construction included the \*\*\*stII\*\*\*

\*\*\*signal\*\*\* \*\*\*sequence\*\*\* to direct secretion to the periplasmic space and one synthetic (Z) domain of Staphylococcal protein A to allow affinity purification using IgG Sepharose. Kistrin was cleaved from the Z-domin by site-specific proteolysis using a mutant subtilisin BPN' and purified by reverse-phase HPLC. This approach facilitated the rapid purification of a set of 43 alanine replacement mutants whose relative affinity for GP IIb-IIIa was measured by competition with immobilized kistrin and by inhibition of platelet aggregation in human platelet-rich plasma. Alanine replacements at R49, G50, and D51 led to weaker

of platelet aggregation by 90-fold, 2-fold, and gt 200-fold, respectively. The conservative D51E mutant was still gt 100-fold less potent whereas R49K had a minor effect (1.8-fold), implying the critical nature of the aspartate for high affinity binding. However, mutations outside of the

region led to proteins indistinguishable from kistrin, suggesting no substantial secondary binding interactions. Furthermore, reduced kistrin is not active. We therefore propose that a favorable conformation of the RGD region alone is responsible for the high affinity binding of kistrin to GP IIb-IIIa.

L7 ANSWER 3 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL

ABSTRACTS INC.

ACCESSION NUMBER: 1991;203774 BIOSIS

DOCUMENT NUMBER: BA91:106999

TITLE: CONSTRUCTION EXPRESSION AND PURIFICATION

OF RECOMBINANT

KRINGLE 1 OF HUMAN PLASMINOGEN AND ANALYSIS

OF ITS

as

INTERACTION WITH OMEGA AMINO ACIDS.

AUTHOR(S): MENHART N; SEHL L C; KELLEY R F;

CASTELLINO F J

CORPORATE SOURCE: DEP. CHEM. BIOCHEM., UNIV. NOTRE DAME, NOTRE DAME, INDIANA

46556.

BIOCHEMISTRY, (1991) 30 (7), 1948-1957. SOURCE:

CODEN: BICHAW. ISSN: 0006-2960.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB An Escherichia coli expression vector, containing the alkaline phosphatase

promoter and the \*\*\*stII\*\*\* heat-stable enterotoxin \*\*\*signal\*\*\*

\*\*\*sequence\*\*\*, along with the cDNA of the kringle I (KI) region of human plasminogen (HPg), has been employed to express into the periplasmic

space amino acid residues 82-163 (E163 .fwdarw. D) of HPg. This region of

the molecule contains the entire K1 domain (residues C84-CI62) of HPg,

well as two non-kringle amino-terminal amino acids (S82-E83) that are present in their normal locations in HPg and a carboxyl-terminal amino acid, D163, that results from mutation of the E163, normally present at this location in the HPg amino acid sequence. After purification of r-K1 by chromatographic techniques, we have investigated its .omega.-amino

binding properties by titration calorimetry, intrinsic fluorescence, and differential scanning microcalorimetry (DSC). The antifibrinolytic agent, epsilon.-aminocaproic acid (EACA), possesses a single binding site for r-K1. The thermodynamic properties of this interaction, studied by